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(54) Modifiable detergent compounds and their use in assays

(57) A method for temporarily increasing the hydrophilic properties of a material, such as a microbial antigen or antibody thereto, comprises contacting the material with a detergent compound containing a modifiable group and subsequently modifying said group (e.g. by cleavage, oxidation or reduction) by addition of a suitable reagent so as to decrease or destroy the detergent properties of said compound. The method may be used to prepare an analyte for assay, subsequent removal of the detergent being made more efficient or even eliminated by means of the modifying reagent. Detergent compounds of general formula $\text{CH}_3(\text{CH}_2)_n - \text{A} - (\text{CH}_2)_m \text{OW}$ (or their salts) where A is $-\text{S}-$ or $-\text{S}-\text{CH}=\text{CH}-$; W is H or SO_3H ; and n is 2-4 and m is 6-11 when A is $-\text{S}-$ or n and m are each 1-10 when A is $-\text{S}-\text{CH}=\text{CH}-$ are also claimed.

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10 COMPOSITIONS AND METHODS FOR REMOVAL OF DETERGENTS

15 Detergents are used throughout industry for a wide
range of applications. They not only have home use for
cleaning eating ware, floors, clothes, etc., but are used
commercially for cleaning manufactured items, in motor
20 fuels, for removal of paints, oil recovery, ore
processing, chemical processing, releasing plastic parts
from molds, suspension of pigments, insecticides and
herbicides, and for use in emulsion polymerization,
dissolution of drugs, purification of proteins,
bioanalytical applications, etc.

25 In assays for analytes of interest, it is often
necessary to solubilize a microorganism to expose its
antigenic sites to recognition by a labelled binding
partner. Sometimes a detergent is used to solubilize the
microorganism. However, the detergent can interfere with
30 the assay; and, frequently, it is desirable to remove a
detergent after it has served its function in assays for
microbial analytes. Removal of the detergent generally
has some drawbacks. One drawback is that the removal

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often requires at least one extra processing step. Another drawback is that material is sometimes lost in the extra processing step. Since the micro-organism is present in small quantities, this can be a serious drawback.

Destructible surfactants have been employed in preparative chemistry. Such surfactants have been used to overcome the effects of emulsion formation during extraction procedures in surfactant-based organized media containing micelles, inverse micelles, and microemulsions. After the preparative reaction has taken place, the destructible surfactant is converted to nonsurfactant products under mild conditions.

Jaeger, et al. in J. Org. Chem. (1986) 51:3956-3959 describe the preparation and characterization of base-sensitive destructible surfactants. Destructible surfactants based on a ketal group are disclosed by Jaeger, et al., ibid. (1984) 49:4545-4547. The preparation and characterization of double-chain destructible surfactants and derived vesicles is discussed by Jaeger, et al., JOACS (1987) 64(11): 1550-1551.

According to a first aspect of the invention, a method is disclosed for temporarily increasing the hydrophilic properties of a material. The method comprises: (a) contacting the material in an aqueous medium with a compound having detergent properties -- the compound comprising a lipophilic portion (containing a modifiable group) and a hydrophilic portion -- whereupon the hydrophilic properties of the material are increased;

and (b) modifying the modifiable group with a modifying reagent, whereupon the detergent properties are modified, and the hydrophilic properties of the material reduced. The detergent is a compound having the structural formula L-J, where L is a lipophilic moiety Y-A-Z capable of modification by the modifying reagent, in which:

Y is alkyl, alkenyl, or alkynyl, having from 2 to 12 carbon atoms; or aralkyl, aralkenyl or aralkynyl, having from 7 to 12 carbon atoms;

A is -S-; -Se-; -CHX-, -CH(X)CH=CH-, wherein X is Cl, Br, or I; -O-CH=CH-; -S-CH=CH-; or -S-S-; and

Z is saturated or unsaturated alkylene having from 1 to 10 carbon atoms or saturated or unsaturated aralkylene having from 7 to 10 carbon atoms;

and J is a hydrophilic moiety.

According to a second aspect of the invention, a method is disclosed for modifying the detergent properties of a detergent in an aqueous medium. This method comprises the step of contacting the medium with a modifying reagent in order to chemically modify the detergent.

According to a third aspect of the invention, an improvement is disclosed in a method for reversibly wetting or dispersing a substance in an aqueous medium, which method comprises contacting the substance with a detergent and removing the detergent. The improvement comprises: (a) employing as the detergent, a compound having the structural formula L-J, wherein L is a lipophilic moiety containing a modifiable group, and J is a hydrophilic moiety; and (b) contacting the combination of the substance and the detergent with a sufficient quantity of a modifying reagent to modify substantially

all of the modifiable group of the detergent whereby the detergent loses its detergent properties.

5 According to a fourth aspect of the invention, a method is disclosed for preparing an analyte for an assay. The method comprises the steps of:

(a) solubilizing the analyte with a detergent of the structural formula L-J, wherein L is a lipophilic moiety containing a modifiable group, and J is a hydrophilic moiety; and (b) modifying the properties of the
10 detergent by the addition of a modifying reagent capable of modifying the modifiable group.

According to a fifth aspect of the invention, a kit is disclosed for conducting an assay for determining an analyte in a sample suspected of containing the analyte.
15 The kit comprises, in packaged form:

a compound having detergent properties and having the structural formula Y-A-Z-J, wherein:

wherein Y is alkyl, alkenyl, or alkynyl,
20 having from 2 to 12 carbon atoms; or aralkyl, aralkenyl or aralkynyl, having from 7 to 12 carbon atoms;
A is -S-, -Se-, -CHX-, -CH(X)CH=CH-, wherein X is Cl, Br, or
I; -O-CH=CH-; -S-CH=CH-; -S-S-;
25 -SO₂CH₂CH₂O-; a ketal group; or
-Si(R)₂O- and -OSi(R)₂O- wherein R is hydrogen or lower alkyl;
Z is saturated or unsaturated alkylene having
30 from 1 to 10 carbon atoms or saturated or unsaturated aralkylene having from 7 to 10 carbon atoms; and
J is a hydrophilic moiety; and

a modifying reagent capable of modifying the
35 detergent properties of the compound Y-A-Z-J.

According to a sixth aspect of the invention, a kit is disclosed for conducting an assay for determining an analyte in a sample suspected of containing the analyte, the analyte being a member of a specific binding pair consisting of a ligand and its complementary receptor. The kit comprises in packaged form:

(1) a detergent having the structural formula Y-A-Z-J, wherein:

wherein Y is alkyl, alkenyl, or alkynyl, having from 2 to 12 carbon atoms; or aralkyl, aralkenyl or aralkynyl, having from 7 to 12 carbon atoms;

A is -S-, -Se-, -CHX-, -CH(X)CH=CH-, wherein X is Cl, Br, or I; -O-CH=CH-; -S-CH=CH-;

-S-S-; $\text{SO}_2\text{CH}_2\text{CH}_2\text{O}-$; a ketal group;

or $-\text{Si}(\text{R})_2\text{O}-$, $-\text{OSi}(\text{R})_2\text{O}-$

wherein R is independently H or lower alkyl;

Z is saturated or unsaturated alkylene having from 1 to 10 carbon atoms or saturated or

unsaturated aralkylene having from 7 to 10 carbon atoms; and

J is OSO_3H , SO_3H , COOH , $\text{PO}(\text{OR})\text{OH}$, or

$\text{OPO}(\text{OR})\text{OH}$, wherein R is H or lower alkyl; and

(2) a member of the specific binding pair complementary to the analyte.

According to a seventh aspect of the invention, a composition is disclosed. The composition includes an aqueous medium containing a microbial antigen and/or its complementary antibody, and a compound of the formula Y-A-Z-J, wherein:

Y is alkyl, alkenyl, or alkynyl, having from 2 to 12 carbon atoms; or aralkyl, aralkenyl or aralkynyl, having from 7 to 12 carbon atoms;

A is -S-; -Se-; -CHX-, -CH(X)CH=CH-, wherein X is Cl, Br, or I; -O-CH=CH-; -S-CH=CH-; -S-S-; -SO₂CH₂CH₂O; a ketal group; or -Si(R)₂O-,
-OSi(R)₂O- wherein R is hydrogen or lower alkyl;

Z is saturated or unsaturated alkylene having from 1 to 10 carbon atoms or saturated or unsaturated aralkylene having from 7 to 10 carbon atoms; and

J is a hydrophilic moiety.

According to an eighth aspect of the invention, a compound is disclosed having the formula

CH₃(CH₂)_n-A-(CH₂)_mOW, wherein A is -S- or -S-CH=CH-; W is H or SO₃H; and n is an integer of 2 to 4, and m is an integer of 6 to 11 when A is -S-, or n and m are independently 1-10 when A is -S-CH=CH-.

The present invention provides a method of chemically changing the properties of a detergent in order to more efficiently remove it or eliminate the need for removal. Before proceeding further with a description of the invention, the following terms are defined.

Alkyl, alkenyl, alkynyl, aralkyl, aralkenyl, and aralkynyl all refer to hydrocarbon chain radicals having a specified number of carbon atoms, up to four carbon atoms if the term "lower" precedes the radical name, or if there is no specification of chain length, having up to 12 carbon atoms:

Accordingly, alkyl means a branched or unbranched saturated hydrocarbon radical such as methyl, ethyl, n-propyl, isopropyl, n-butyl, iso-butyl, sec-butyl, tert-butyl, pentyl, hexyl, heptyl, octyl, nonyl, decyl, undecyl, dodecyl, etc., including positional isomers.

Alkenyl refers to any branched or unbranched unsaturated carbon chain radical having the number of carbon atoms specified, or up to 12 carbon atoms if no limitation on the number of carbon atoms is specified. Accordingly, alkenyl radicals have 1 or more double bonds between carbon atoms, e.g., vinyl, allyl, 1-propenyl, 2-butenyl, 1,3-butadienyl, 2-pentenyl, etc., and are otherwise of similar scope to alkyl radicals, defined above.

Alkynyl refers to hydrocarbon radicals of the scope of alkenyl, but having 1 or more triple bonds in the radical, e.g., ethynyl, 2-propynyl, 2-penten-4-ynyl, etc.

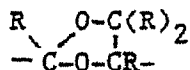
Aralkyl of 7 to 12 carbon atoms refers to a radical having an alkyl group to which is attached a benzene ring, such as the benzyl radical, phenethyl radical, 3-phenylpropyl radical, and the like.

Aralkenyl and aralkynyl are of similar scope, except that an alkenyl or alkynyl group replaces the alkyl group to which the benzene ring is attached. Examples of aralkenyl and aralkynyl are styryl and benzylidyne, respectively.

A ketal group refers to the radical



wherein R is H or lower alkyl, and includes cyclic ketals of the formula



Saturated or unsaturated alkylene having from 1 to 10 carbon atoms means, for the purposes of this invention, a hydrocarbon chain (a radical) that is part of a molecule (in this context, part of a detergent or compound having detergent properties), and that is situated such that it bridges two other parts of the

molecule. Specifically, in the formula Y-A-Z-P described herein, Z can be alkylene, such as methylene ($-\text{CH}_2-$), ethylene ($-\text{CH}_2-\text{CH}_2-$), and the like. For the purposes of this invention, however, the term alkylene is used for corresponding unsaturated radicals, such that $-\text{CH}=\text{CH}-$ for example, would be understood to be within the scope of "unsaturated alkylene." Similarly, saturated or unsaturated aralkylene is a carbon chain having a benzene ring as a side chain in place of a hydrogen atom, such that $-\text{CH}(\text{C}_6\text{H}_5)-$ would be an example of saturated aralkylene.

An analyte is a compound or composition to be measured, which is usually a specific binding pair member (defined below) and may be a ligand or a receptor.

In accordance with the definitions provided below, the material whose hydrophilic properties are increased may be an antigen and/or an antibody, most preferably, a chlamydia antigen, a chlamydia antibody, a gonococcus antigen, or a gonococcus antibody.

An antigen is any substance to which an organism can elicit an immune response. Antigens are generally capable of binding specifically to complementary antibodies. Antigens may be any organic group and will normally be poly(amino acids), i.e., polypeptides and proteins, polysaccharides lipopolysaccharides, glycoproteins, lipoproteins, ribonuclear proteins, and the like. The antigens will frequently be components of bacteria, viruses, mitochondria, nuclei, cell membranes and the like. In this context:

Polysaccharides are a combination of at least about three or four monosaccharides, linked together by glycosidic bonds. Polysaccharides include, for example, starch glycogen, cellulose and dextran.

A wide variety of proteins may be considered: the family of proteins having similar structural

features, proteins having particular biological functions, proteins related to specific microorganisms, particularly disease causing microorganisms, etc.

5 Lipopolysaccharides (LPS) are molecules containing lipid and polysaccharide moieties and are present in the outer layer of the cell membranes of gram-negative bacteria.

10 Bacteria include gram-negative bacteria, including but not limited to, Chlamydia trachomatis, Chlamydia psittaci, E. coli, Pseudomonas fluorescens, Azotobacter vinelandii, Proteus vulgaris, Hydrogenomonas sp., Aerobacter aerogenes,
15 Neisseria gonorrhoeae, Treponema pallidum, Serratia marcescens, Achromobacter fischer, Bacillus subtilis, Bacillus megaterium, Sarcina lutea, Micrococcus pyogenes, Lactobacillus casei, Torulopsis utilis and Streptomyces griseus,
20 Shigella, Campylobacter, Salmonella, Legionella, Hemophilus influenza, and so forth.

A microbial antigen is an antigen that is present in a host that is in a disease state, where the disease is caused by a microbe such as a
25 bacteria (examples of such diseases are gonorrhoeae and chlamydia).

For the most part, the antigens employed in the subject invention will have a molecular weight of at least about 1,000, more usually at least about 2,500, frequently
30 greater than 5,000. In the poly(amino acid) category, the poly(amino acids) of interest will generally be from about 5,000 to 5,000,000 molecular weight, more usually from about 20,000 to 1,000,000 molecular weight. The precise nature of some of the antigens together with
35 numerous examples thereof are disclosed in U.S. Patent No. 4,299,916 to Litman, et al., particularly at columns 16 to 23.

An antibody is a molecule, i.e., an immunoglobulin, formed by the immune system of a host in response to exposure to an antigen. Generally, an antibody specifically binds to, and is accordingly defined as complementary to, the antigen. The antibody can be monoclonal or polyclonal and can be prepared by techniques that are well known in the art such as immunization of a host and collection of sera (polyclonal) or by preparing continuous hybrid cell lines and collecting the secreted protein (monoclonal). Antibodies may include a complete immunoglobulin or fragment thereof, which immunoglobulins include the various classes and isotypes, such as IgA, IgD, IgE, IgG1, IgG2a, IgG2b and IgG3, IgM, IgG1, IgG2, IgG3, IgG4 etc. Fragments thereof may include Fab, Fv and F(ab')₂, Fab', and the like. When it is recited herein that a solution or material comprises an antigen or an antibody, the solution or material can comprise both the recited antigen and the recited antibody.

An assay kit is a product, having reagents in packaged combination, used to determine the presence of an analyte in a sample. The reagents are packaged to insure shelf-life, for example, each in separate containers in instances where co-reactivity would destroy shelf life if the reagents were packaged together.

A detergent, or a compound having detergent properties, refers to a compound that can, upon contact with another material, change the hydrophilic properties of that other material. Generally, a detergent increases these hydrophilic properties. Accordingly, a detergent or a compound having detergent properties increases the contacted material's ability to absorb or adsorb water. The detergents according to this invention have a hydrophilic moiety, such as an acidic group or a salt thereof, and a lipophilic moiety, containing a modifiable

group. The modifiable group has an "unmodified state" and a "modified state". In the unmodified state, the detergent or compound having detergent properties has the effect of increasing the hydrophilic properties of the material with which it is in contact. In the modified state, the detergent properties are decreased, such that the hydrophilic properties of the contacted material are reduced.

A modifying reagent is a reagent that chemically modifies the detergent, or the compound having detergent properties, thereby changing the modifiable group from the unmodified to the modified state. There are several ways that the modifying reagent can act upon the modifiable group in the detergent:

For example, the modifying reagent can act by cleaving the detergent at or near the modifiable group. For the purposes of this invention, "cleaving" means splitting the detergent molecule at one or more bonds of any type (accordingly, the term is to be broadly construed, to include dissociation into one or more fragments). Examples of nucleophilic modifying reagents that can cleave or add to the detergent at a modifiable group are: fluoride, thiourea (i.e., $\text{NH}_2\text{-CS-NH}_2$); hydroxylamine (NH_2OH); hydrazines (i.e., compounds containing the R-NH-NH_2 group, where R is hydrogen or alkyl of 1 to 4 carbon atoms); hydrazides (i.e., R-CO-NH-NH_2 where R is alkyl of 1 to 4 carbon atoms); ammonia; mercaptans (lower alkanethiols), such as ethyl mercaptan (ethanethiol: $\text{CH}_3\text{CH}_2\text{SH}$); mercapto alcohols such as dithioerythritol and mercapto acids such as HS-R-COOH where R is alkyl up to 4 carbon atoms; thiosulfates (i.e., $\text{M}_2(\text{S}_2\text{O}_3^-)_n$ where M is a cation with n positive charges); and thioacids

(i.e., R-CO-SH, R-CS-OH or R-CS-SH where R is alkyl of 1 to 4 carbon atoms). The foregoing nucleophilic reagents could, for example, cleave silylethers, disulfides, esters, ketones or Schiff bases, or enter into nucleophilic substitution reactions such as, for example, displacement of halides in alkyl halides, allylic halides, or 3-haloalkynes, typically allylic chlorides. The modifying reagent can be an anion selected from the group consisting of anions containing sulfur such as sulfide, etc., oxygen such as hydroxide, alkoxide, aralkoxide, etc., or nitrogen such as azide, ammonia, etc.

Additionally, the modifying reagent can act by oxidizing the modifiable group. Suitable oxidants are hypochlorite and hypobromite. Additionally, peroxides and peracids are good oxidizing agents, such as lower alkyl or acyl hydroperoxides, H_2O_2 , perborate, persulfate, and peracetate. Such oxidizing reagents could oxidize many types of modifiable groups, such as seleno or thioethers, seleno or thio vinyl ethers, sulfoxides, or enolethers.

Yet another mode of action for a modifying agent is by reducing the modifiable group. Suitable reducing agents are sulfite salts, hyposulfite salts, and mercaptans, particularly C3 and C4-bis mercapto alkanes and alkanols which can reduce disulfide bonds.

Preferably, when the modifiable group A is -CH(X)-CH=CH-, the modifying reagent is an anion selected from the group consisting of anions containing sulfur, oxygen, or nitrogen. Also, when A is -S-S-, said modifying reagent is preferably a lower alkyl or acyl mercaptan, an oxidizing agent or a hyposulfite salt.

A specific binding pair (sbp) consists of two different molecules (first and second sbp members), where one of the molecules has an area on the surface or in a cavity which specifically binds to a particular spatial and polar organization of the other molecule. The two members of a specific binding pair are referred to as a ligand and its complementary receptor (antiligand).

The present invention is generally directed to a method for removal of detergents by chemically modifying the detergent to modify or destroy its detergent properties. Usually the method will be useful for detergents used in an aqueous medium. The method uses modifying reagents which will not damage the material to be freed of detergent, and which yield more than mere protonation or deprotonation of the detergent. Reducing agents and changes in pH can be used. Usually a mild oxidant or a nucleophile is used. Preferred oxidants are peroxides and peracids. Preferred nucleophiles are thiosulfates, mercaptoacids, thiourea, hydroxylamines, ammonia, hydrazines, hydrazides, etc. Preferred reducing agents are dithioerythritol, dithiothreitol, ascorbic acid, borohydride, etc.

Detergents are used that, on reaction with these modifying reagents, markedly change in polarity or are cleaved. Normally a nonpolar modifiable group that enhances or does not eliminate the detergent properties is incorporated into a detergent. The modifiable group will preferably be located to maximally interfere with the detergent properties when it is modified. This group will normally be situated in the lipophilic portion of the detergent, preferably near the center of the lipophilic portion or distal from the center and the hydrophilic group or, if more than one group is used, spaced to provide segments of greatly reduced lipophilic properties.

Accordingly, a typical formula for a detergent used in this invention would be L-J, where L is a lipophilic moiety containing a modifiable group, and J is a hydrophilic moiety. Preferably, the lipophilic moiety has the formula Y-A-Z. In this formula, Y is a saturated or unsaturated aliphatic hydrocarbon having from 2 to 12 carbon atoms, or is aralkyl, aralkenyl or aralkynyl of up to 12 carbon atoms. A is a modifiable group, such as -S-; -Se-; -CHX- or -CH(X)-CH=CH- where X is Cl, Br, or I; -O-CH=CH-; -S-CH=CH- or -S-S-. Z is saturated or unsaturated alkylene having from 1 to 10 carbon atoms or saturated or unsaturated aralkylene having from 6 to 10 carbon atoms. Preferably, Z has from 2 to 10 carbon atoms, especially when A is not -O-CH=CH- (the latter A already provides 2 carbon atoms). J is preferably OSO_3H , SO_3H , COOH , PO(OR)OH , or OPO(OR)OH , wherein R is H or lower alkyl.

Preferably, detergent compounds used in the methods, compositions and kits of this invention have the formula Y-A-Z-J, which may be $\text{CH}_3(\text{CH}_2)_n\text{-S-(CH}_2)_m\text{OSO}_3\text{H}$, or a salt thereof, wherein n is an integer of 1 to 6, preferably 2 to 4, and m is an integer of 4 to 11, preferably 6 to 11. Most preferably Y-A-Z-P is $\text{CH}_3(\text{CH}_2)_2\text{-S-(CH}_2)_{11}\text{OSO}_3\text{H}$ or $\text{CH}_3(\text{CH}_2)_4\text{-S-(CH}_2)_6\text{OSO}_3\text{H}$, or a salt thereof.

The detergent must be capable of chemical modification upon addition of a modifying reagent. There are many ways of carrying out such a chemical modification, including, but not limited to, cleaving, oxidizing, and reducing the modifiable group. Cleaving can be accomplished, for example, with the use of a nucleophile, such as a fluoride, a thiosulfate, a mercaptan, a thioacid, a thiourea, a hydroxylamine, a hydrazine, a hydrazide or ammonia. Oxidizing can be accomplished, for example, with a peroxide or a peracid

such as a lower alkyl or acyl hydroperoxide, H_2O_2 ,
perborate or persulfate; or by using hypochlorite or
hypobromite. Reducing can be accomplished, for example,
by using a sulfite salt, a hyposulfite salt, or a lower
5 alkyl or lower acyl mercaptan.

Of course, the modifiable group must be one that can
be acted upon by the modifying reagent. For example,
when an acid is used as a modifying reagent to disrupt
the detergent, the modifiable group can be an enolether
10 or acetal. Alternatively, the modifiable group can be a
Schiff base or enamine.

When a reducing agent is used as a modifying
reagent, such as a mercaptan, the modifiable group can be
a disulfide.

15 When a nucleophile is used as a modifying reagent,
the modifiable group can be a Schiff base or ketone, but
will preferably be chloride, bromide, or iodide,
preferably an allylic halide or a 3-haloalkyne, usually
an allylic chloride or, when the nucleophile is fluoride,
20 the modifiable group may be a silyl ether.

When an oxidant is used as a modifying reagent, the
modifiable group can be a seleno or thio ether or a
seleno or thio vinyl ether.

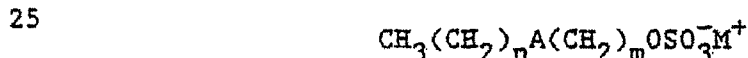
25 As compounds per se, the detergents of this
invention have the formula

$CH_3(CH_2)_n-A-(CH_2)_mOW$, wherein A is $-S-$ or
 $-S-CH=CH-$; W is H or SO_3H ; and n is an integer of 2 to
4, and m is an integer of 6 to 11 when A is $-S-$, or n and
m are independently 1 to 10 when A is $-S-CH=CH-$, or a
30 salt thereof. Preferably, when A is $-S-$, m is 11 and n
is 2 or m is 6 and n is 4, most preferably, the compounds
are $CH_3(CH_2)_2-S-(CH_2)_{11}OSO_3H$ or
 $CH_3(CH_2)_4-S-(CH_2)_6OSO_3H$, or a salt of either
of the foregoing. Or, when A is $-S-CH=CH-$, preferably m
35 is 6 and n is 4, most preferably, the compound

$\text{CH}_3(\text{CH}_2)_6\text{-S-CH=CH-(CH}_2)_4\text{OSO}_3\text{H}$ or a salt thereof.

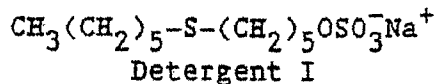
Preferred applications of this invention are for bioanalytical and drug delivery technology. For drug delivery, emulents can be provided that will release a drug by contact with a releasing agent, and liposomes used in drug delivery can be freed of contaminating detergents. For bioanalytic use, latex particles prepared by emulsion polymerization can be freed of detergent. Proteins can be recovered from detergent solutions used in gel electrophoresis. Cell membrane components that are separated by solubilization with detergents can be reconstituted. Antigens can be separated from cellular debris and biological fluids by detergents and then freed of the detergent to permit adsorption on surfaces and/or antibody binding.

The method has particular application to analytical microbiology. Microbial antigens, particularly chlamydia lipopolysaccharide, and gonococcus surface proteins can be solubilized by detergent of the present invention and then released so as to permit analysis by antibody binding. For this purpose, derivatives of alkylsulfate detergents are preferred, having the formula:

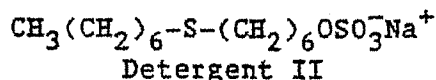


where A can be, for example, -S-, -S-S-, -S-CH=CH-, -Se-, -CHX-, -CH(X)CH=CH- (X=Cl, Br, I), -O-CH=CH-, -SO₂CH₂CH₂O-; -Si(R)₂O-, -OSi(R)₂O-, wherein R is H or lower alkyl; n and m can vary from 1 to 12, and together are at least 5; and M is an alkali metal. More preferably, however, A is -S-; -Se-; -CHX-, wherein X is Cl, Br, or I; -O-CH=CH-; or -S-S-. Most preferably, A is -S-, n is an integer of 1 to 6, and m is an integer of 4 to 11.

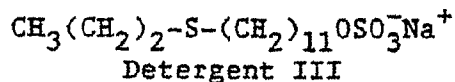
Particularly preferred detergents are:



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Of these, Detergent II is presently the most preferred. These compounds can solublize antigen and then release the antigen when treated with hydrogen peroxide.

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The present invention has application to numerous techniques known for detecting antigens in a sample, such as a biological fluid, i.e., blood, urine, cell cultures. It is important to be able to quickly and accurately detect the presence of antigens such as gram-negative bacteria, i.e., Chlamydia trachomatis, Chlamydia psittaci and Neisseria gonorrhoeae because of the prevalence of diseases associated with such bacteria. Some of the techniques utilized to detect antigens involve cell culture procedures, electrophoresis, and the like.

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Many of the detection techniques involve immunoassays. Some of these techniques involve detecting antibodies to the antigen of interest. It is, however, preferable to assay for antigens rather than antibodies. Immunoassay techniques for the detection of antigens often involve enzyme immunoassays such as the enzyme linked immunosorbent assay generally referred to as ELISA. Such assays typically involve detecting the antigen of interest by coating the antigen on a bare solid surface or a surface that has been pre-coated with

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a protein such as an antibody. After coating the surface of the support with the antigen, the surface is washed to remove unbound antigen. Thereafter, the antigen on the surface is contacted with antibody for the antigen that is labeled or is capable of being labeled. The surface is again washed and the antibody that has bound to the surface of the support is detected by detecting the label.

One method for determining Chlamydia trachomatis antigen in a clinical specimen is disclosed in U.S. Patent No. 4,497,899. The method disclosed involves lysing Chlamydia cells in the specimen to release the antigen; coating a bare solid support with the cell lysate; separating the coated support from the specimen; treating the separated support with antibody to form an antigen-chlamydia antibody complex on the support; separating the complex from unbound antibody; treating the bound complex with labeled antiglobulin to form an antigen-antibody-labeled antiglobulin complex on the support; separating the latter complex from unbound labeled antiglobulin; and determining bound labeled antiglobulin as a measure of antigen in the specimen. U.S. Patent No. 4,497,900 discloses a method for determining Neisseria gonorrhoeae analogous to that in U.S. Patent No. 4,497,899.

An indirect method for determining an antigen in a liquid sample is disclosed in U.S. Patent No. 4,067,959 involving adsorbing antigen of the same immunological type as the sample antigen onto a solid support surface, reacting a known quantity of specific labeled antibody in solution with the sample antigen and with the adsorbed antigen. The labeled antibody is in excess so that a portion of the labeled antibody is bound in the solution to the sample antigen and excess labeled antibody immunologically reacts with the adsorbed antigen on the surface. The surface is washed and then the quantity of

reacted labeled antibody on the surface is determined as a measure of the antigen in the sample.

5 A method for assaying for the presence of a Chlamydial infection involving generating antibodies against the principal outer membrane protein of Chlamydia trachomatis is disclosed in U.S. Patent No. 4,427,782. The method comprises treating a sample suspected of containing chlamydial infection to solubilize the Chlamydia outer cell membrane protein, contacting the
10 generated antibodies with the solubilized specimen and determining the reaction between the antibodies and the solubilized specimen.

Another method for detecting an antigen in a biological sample is disclosed in U.S. Patent Application
15 Serial No. 135,869, filed December 21, 1987, (corresponding to Japanese Patent Application No. 63-321,869; Canadian Patent Application S.N. 586,443 and European Patent Application No. 88312056.0). The method involves providing in combination a solid support,
20 which is substantially free of specific binding proteins, and a medium comprising an antigen from the sample and an antibody for the antigen. The combination is incubated under conditions sufficient for the antibody when bound to the antigen to bind to the support. The presence or
25 amount of antibody on the support or in the medium is determined and is related to the presence of antigen in the sample.

The disclosures of the above patents and patent applications are incorporated herein by reference in
30 their entirety. In many of the above methods solubilization of a microorganism is important or necessary.

Solubilization thereof can be accomplished in accordance with the present invention by incubation of
35 the bacterial sample in the presence of a detergent as

described above, usually in the concentration range of from about 0.01 to 1.0%, weight to volume. Temperatures employed for solubilizing the bacterial sample are usually in the range from about 10° to 50°C, more usually from about 15° to 100°C. For the most part, relatively short times are required for solubilization. Usually, the solubilization can take at least about 5 seconds and not more than 1 hour, more usually about 30 seconds to about 30 minutes. The time is dependent on the nature of the bacterial sample, the antigen, and the type and amount of detergent and temperature used.

The solubilization medium is generally aqueous and may contain up to 40% of an organic solvent. The pH of the solubilization medium will usually be in the range of about 2 to 12, more usually in the range of about 5 to 9. The pH is generally chosen to achieve a high level of solubilization of the antigen. Various buffers may be used to achieve and maintain the pH during the solubilization. Illustrative buffers include borate, phosphate, carbonate, Tris, barbital and the like. The particular buffer employed is not critical but one buffer may be preferred over another.

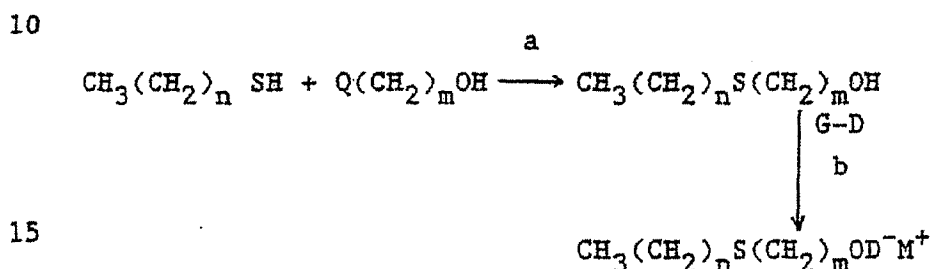
In immunoassays where solid-phase surfaces are employed for the capture of solubilized bacterial components (antigen) and a known detergent such as SDS, Triton, NP-40, sarcosyl, or chenodeoxycholate is used for the solubilization, sample dilution is usually required subsequent to the solubilization step in order to reduce the interference of the detergent on the binding of antigen to the captive surface. The present invention substantially reduces the need for or avoids such a dilution and thereby provides for greater assay sensitivity.

Thioether compounds (A= -S-) for use in the methods, kits, and compositions of the present method can be

prepared, for example, by reacting an appropriate thiol with a haloalcohol under conditions for displacement of the halide by deprotonated thiol to yield a thioether alkanol, which can then be converted to an ester comprising the hydrophilic moiety.

For example, referring to Scheme I:

SCHEME I



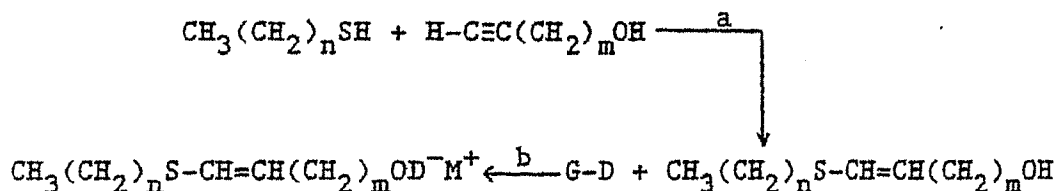
wherein:

- n and m can vary from 1 to 12;
- Q = Cl or Br;
- 20 G = Cl, Br, or Y, where Y = (CH₃CH₂)₃N, (CH₃)₃N, or pyridine;
- D = SO₃, PO(OR)O, wherein R is H or lower alkyl, ClC(O)NHCH₂CH₂SO₃, or ClC(O)CH₂CH₂COO
- 25 M = Na or K
- a = metal hydride such as sodium hydride in an ether solvent such as ethyl ether, tetrahydrofuran, etc.; and
- 30 b = aqueous alkali such as sodium hydroxide, potassium hydroxide, etc.

Modifiable detergents containing a selenium group (A = -Se-) for use in the present invention can be prepared in a manner similar to that described above for the destructible detergents containing a thioether linkage.

Vinyl thioether detergents (A = -S-CH=CH-) for use in the method, kits, and compositions of the present invention can be prepared, for example, by reacting an appropriate thiol with an appropriate alkynyl alkanol under conditions wherein the thiol moiety adds to the alkynyl group. The resulting vinyl thioether alkanol is then converted to an ester comprising the hydrophilic moiety. For example, referring to Scheme II:

SCHEME II



wherein:

n and m can vary from 1 to 12;

M = Na or K;

G = Cl or Br or Y, wherein Y = (CH₃CH₂)₃N, (CH₃)₃N, or pyridine;

D = SO₃, PO(OR)O wherein R is H or lower alkyl,

ClC(O)NECH₂CH₂SO₃, ClC(O)CH₂CH₂COO, etc;

a = diacyl peroxide such as dibenzoyl peroxide, etc. in an alkane (C₅-C₈) solvent such as heptane, etc.; and

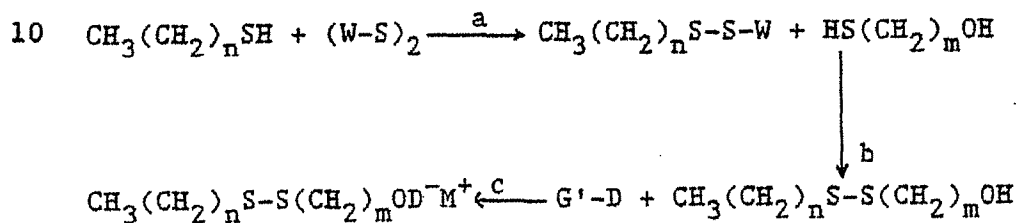
b = aqueous alkali such as sodium hydroxide, potassium hydroxide, etc.

Disulfide detergents (A = -S-S-) for use in the present invention can be prepared, for example, by reacting an appropriate thiol with an appropriate activated halosulfide under conditions for displacement of the activating group by the thiol group to form an activated disulfide, which can be

reacted with an appropriate thiol alkanol. The resulting disulfide alkanol can then be treated to convert the alcohol moiety to an ester comprising the hydrophilic moiety. For example, referring to Scheme III:

5

SCHEME III

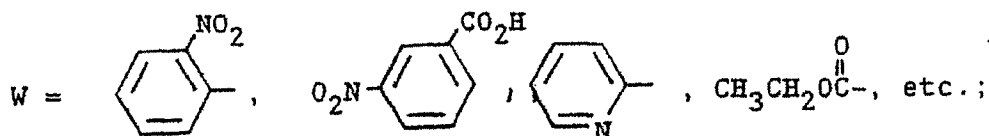


wherein:

n and m can vary from 1 to 12;

20 $\text{G}' = \text{Br}, \text{Cl}, \text{ or } \text{Y} \text{ where } \text{Y} = \text{CH}_3\overset{\text{O}}{\parallel}\text{C}-\text{S},$
 $\text{NO}_2-\text{C}_6\text{H}_4\text{S}-, \text{ 2-pyridylthio, etc.};$
 $\text{D} = \text{SO}_3, \text{ PO(OR)O where R is H or lower}$
 $\text{alkyl, ClC(O)NHCH}_2\text{CH}_2\text{SO}_3, \text{ ClC(O)CH}_2\text{CH}_2\text{COO,}$
 $\text{etc.};$
 $\text{M} = \text{K or Na};$

25



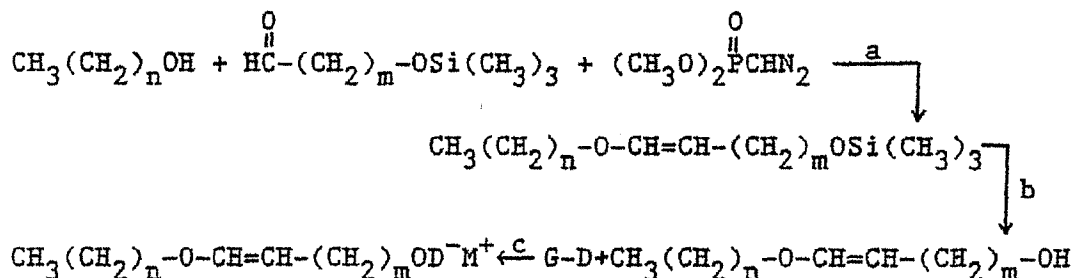
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a = tri(lower)alkylamine, such as triethylamine in ether
 such as ethyl ether, tetrahydrofuran, etc.;
 b = a; and
 c = aqueous alkali such as sodium or potassium hydroxide,
 etc.

35

Vinyl ether detergents (A= -O-CH=CH-) for use in the methods, kits, and compositions of the present invention can be prepared, for example, by reaction of an appropriate alkanol with an appropriate tri(lower)alkyl silylated alcohol aldehyde and a diazomethyl lower alkyl phosphate under strongly basic conditions to give a vinyl ether with a terminal silyl group, which can be converted to an ester comprising the hydrophilic moiety. For example, referring to Scheme IV:

SCHEME IV



wherein:

n and m can vary from 1 to 12;

G = Cl, Br, or Y where Y = (CH₃CH₂)₃N,
(CH₃)₃N,

or pyridine;

D = SO₃, PO(OR)O where R=H or lower alkyl,
ClC(O)NHCH₂CH₂SO₃, ClC(O)CH₂CH₂COO,

etc.;

M = Na or K;

a = metal salt of lower alkyl alcohol, such as
tert-butyl alcohol, etc.;

b = metal halide such as sodium fluoride, etc.; and

c = aqueous alkali such as sodium or potassium
hydroxide, etc.

Haloalkyl detergents (A = -CHX- or -CH(X)-CH=CH-) for use in the present invention can be prepared from the corresponding alkenyl alcohol by reaction with a haloacid. The alcohol moiety of the resulting haloalkyl alkanol is then converted to a hydrophilic moiety. For example, referring to Scheme V:

SCHEME V

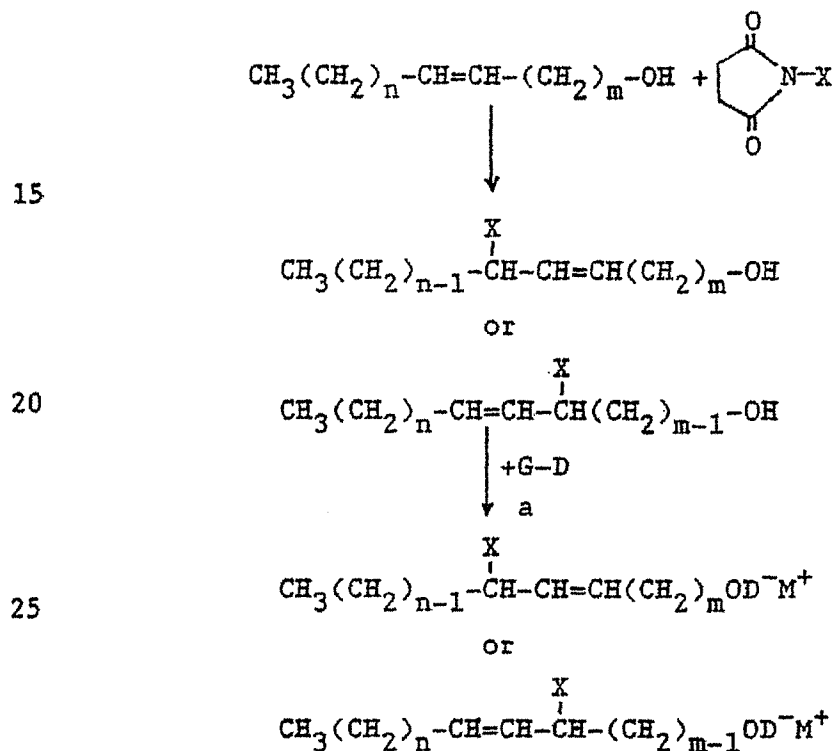
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wherein:

n and m can vary from 1 to 12;

X = Cl, Br, or I;

G = Cl, Br, or Y where Y = (CH₃CH₂)₃N, (CH₃)₃N, or pyridine;

35

D = SO₃, PO(OR)O wherein R is H or lower alkyl ClC(O)NHCH₂CH₂SO₃, or ClC(O)CH₂CH₂COO; and

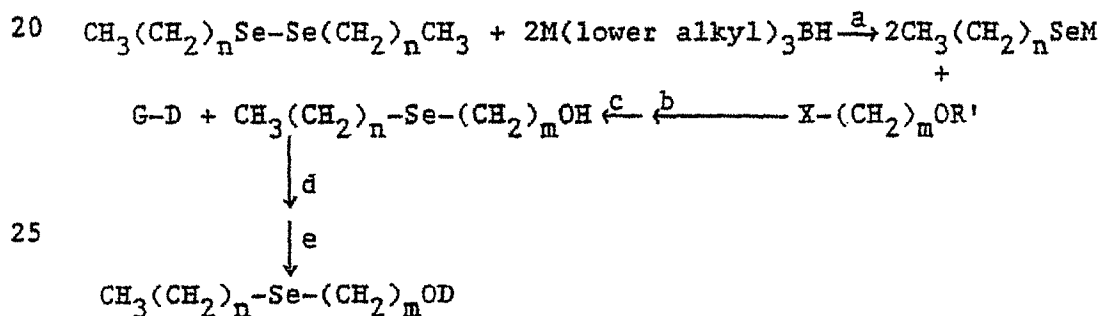
M = K or Na;

a = aqueous alkali such as sodium or potassium hydroxide, etc.

5 The detergents wherein A is -CHX- may be prepared in a similar manner, by additionally reducing the double bond of -CH(X)-CH=CH-.

Modifiable detergents containing a selenium group (A = -Se-) for use in the present invention can also be prepared by reacting a symmetrical dialkyl diselenide with a metal trialkylborohydride in an ether solvent to give a alkyl metal selenolate, which can be reacted with an appropriate haloalcohol. The alcohol moiety of the resulting seleno-alkanol can then be esterified with the appropriate hydrophilic moiety. For example, referring to Scheme VI:

SCHEME VI



wherein:

30 n and m can vary from 1 to 12;
M = a metal such as lithium, etc.;
R' = alcohol protecting group such as $(\text{CH}_3)_3\text{Si}$ -, etc.;
X = Cl, Br or I;
35 G = Cl, Br, or Y where Y = $(\text{CH}_3\text{CH}_2)_3\text{N}$, $(\text{CH}_3)_3\text{N}$ or pyridine;

D = SO_3H , $\text{PO}(\text{OR})\text{OH}$, where R is H or lower alkyl
 $\text{ClC}(\text{O})\text{NHCH}_2\text{CH}_2\text{SO}_3\text{H}$, $\text{ClC}(\text{O})\text{CH}_2\text{CH}_2\text{COOH}$;

a = ether solvent such as ethyl ether,
tetrahydrofuran, etc.;

5 b = same as a;

c = agent for removing a protecting group from an
alcohol, such as $(\text{CH}_3)_4\text{N}^+\text{F}^-$, etc.;

d = aqueous alkali, such as sodium or potassium
hydroxide, etc.; and

10 e = aqueous acid, such as hydrochloric and sulfuric
acid, etc.

All of the above compounds can be prepared as a
metal salt of the hydrophilic group such as the sodium
salt, potassium salt and the like.

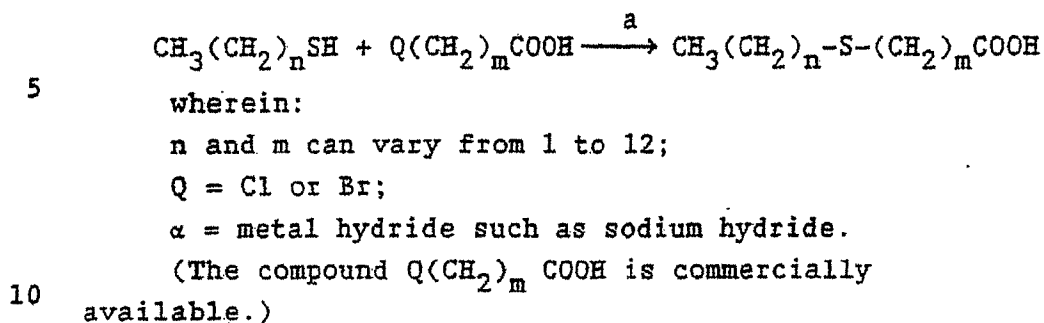
15 Other detergents ($\text{A} = -\text{SO}_2\text{CH}_2\text{CH}_2\text{O}-$, a ketal
group, or $-\text{Si}(\text{R})_2\text{O}-$ wherein R is lower alkyl) for use
in the assay methods, kits, and compositions of the
present invention can be prepared according to procedures
described by Jaeger, *et al.*, *supra*. The detergents
20 wherein A is $-\text{OSi}(\text{R})_2\text{O}-$ may be prepared in an analogous
fashion.

The above noted preparation schemes describe methods
to prepare the sulfate ($-\text{OSO}_3-\text{Na}^+$) and phosphate
($-\text{OPO}(\text{OR})\text{O}^-\text{Na}^+$) salts. The sulfonic, phosphonic and
25 carboxylic acid salts may be prepared as follows:

Carboxylic acids and their salts ($\text{J} = -\text{COOH}$ or
 $-\text{COO}^-\text{Na}^+$) for use in the methods, kits and
compositions of the present invention can be prepared,
for example, by reacting an appropriate thiol with an
30 appropriate halo carboxylic acid, under conditions for
displacement of the halide by deprotonated thiol to yield
a thioether carboxylic acid. For example, referring to
Scheme VII:

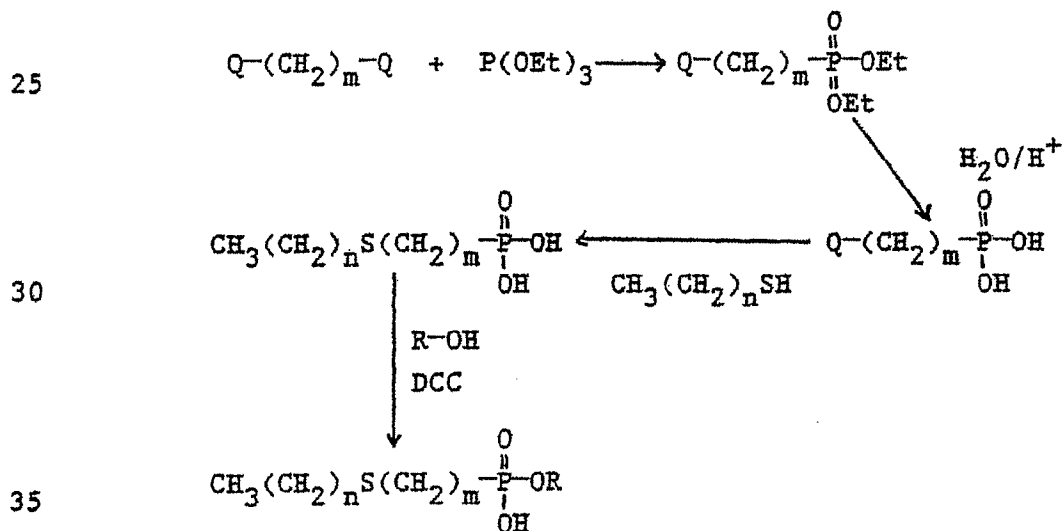
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SCHEME VII



Phosphonic acid salts ($\text{J}=\text{PO}(\text{OR})\text{O}^-\text{Na}^+$) for use in the methods, kits and compositions of the present invention can be prepared, for example, by reacting an
 15 appropriate dihaloalkane with an appropriate phosphite ester to give the phosphonic ester, which is hydrolyzed to yield the phosphonic acid derivative. Reaction with an appropriate thiol, followed by an alcohol in the presence of dicyclohexylcarbodiimide provides the
 20 thioether phosphonic acid salt, as per Scheme VIII:

SCHEME VIII



wherein:

n and m can vary from 1 to 12:

O = Cl or Br

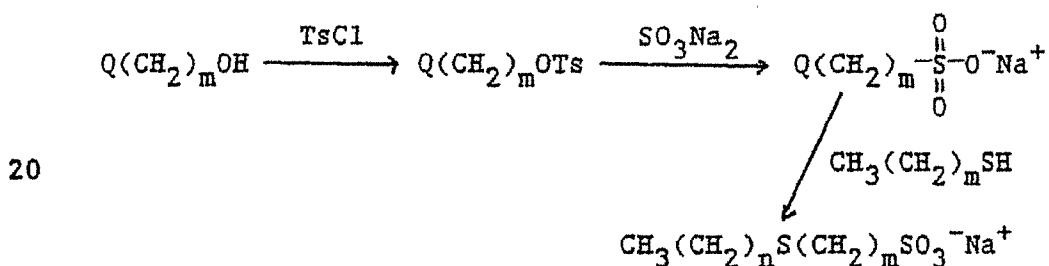
(The starting compounds are commercially available.)

5

10 Sulfonic acid salts ($J = \text{SO}_3^-\text{Na}^+$) for use in the methods, kits and compositions of the present invention can be prepared, for example, by reacting a haloalcohol with tosyl chloride to give a toluene sulfonic acid halo ester. This is subsequently reacted with SO_3Na_2 and an appropriate thiol to give the thio ether sulfonic acid salt, as per the following Scheme IX:

SCHEME IX

15



wherein:

25 n and m an vary from 1 to 12;

0 = Cl or Br: and

TsCl = tosyl chloride.

As mentioned above, the modifying reagent and conditions chosen for modifying the properties of a detergent used in accordance with the present invention vary depending on the particular modifying group. The following reagents and conditions are provided by way of example and not limitation.

35 Nucleophilic modifying reagents and conditions:

(a) β -mercaptoethanol at alkaline pH for disulfide

cleavage, (b) thioacetic acid, (c) hydroxylamine at alkaline pH, (d) hydrazine at alkaline pH, (e) ammonia at alkaline pH, and (f) thiourea at alkaline pH.

Oxidizing modifying agents and conditions:

- 5 (a) hydrogen peroxide in acetate buffer, pH 4.5,
(b) sodium periodate in water, (c) peracetic acid in acetic acid, (d) dichlorophenyl iodide in an organic solvent, (e) tert-butoxy hydrochlorite in water, (f)
10 sodium hypochlorite or hypobromite in water, (g) perborate, sodium borate at alkaline pH, and (h) $\text{Na}_2\text{S}_2\text{O}_8$ in water.

Reductive modifying reagents and conditions:

- (a) dithiothreitol or dithioerythritol, pH 8 for
15 reductive of cleavage of disulfides, (b) triphenylphosphine in aqueous dioxane, (c) n-butylphosphine at alkaline pH, and (d) sodium sulfite at alkaline pH.

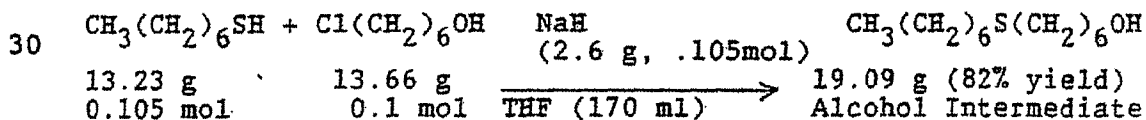
EXAMPLES

- 20 The invention is further demonstrated by the following illustrative examples. Parts and percentages are by weight to volume except where otherwise indicated.

EXAMPLE I

25 PREPARATION OF DEGRADABLE DETERGENT
CONTAINING THIOETHER GROUP

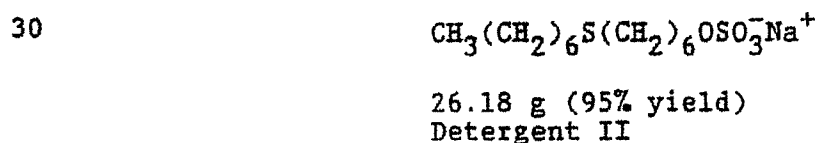
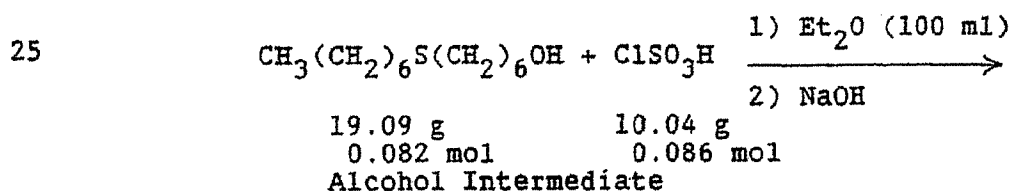
Part A:



1. Heptyl mercaptan in tetrahydrofuran (THF) was added
35 to a vigorously stirred suspension of NaH in THF over 45 minutes. The mixture was stirred at room temperature for one hour under argon.

2. Chlorohexanol in THF was added dropwise to a vigorously stirred suspension of sodium heptyl mercaptide under argon over 30-45 minutes. The mixture was stirred at room temperature under argon for 24 hours. Reaction completion was checked by thin layer chromatography.
3. The THF was evaporated on a rotary evaporator. Water was added, and extraction was performed three times with ethyl acetate (EtOAc). The EtOAc extraction was washed with water. Then the EtOAc extracts were washed with saturated brine, and dried over Na_2SO_4 . The Na_2SO_4 was filtered and evaporated to a yellow oil on a rotary evaporator.
4. The product was chromatographed with a Waters Prep 500 HPLC, using hexane-EtOAc. Appropriate fractions were combined and evaporated to a clear, light yellow oil. The product was distilled under vacuum, boiling point 85-100°C/0.01 mm.

Part B:



1. The Et_2O (ethyl ether) was cooled to 0-5°C with stirring under argon.
- 5 2. ClSO_3H was added dropwise, maintaining temperature at 0-5°C.
3. The Alcohol Intermediate was added dropwise, maintaining temperature at 0-5°C.
- 10 4. Et_2O was removed on rotary evaporator; then Et_2O was added and evaporated again on a rotary evaporator/vacuum pump.
- 15 5. The solution was poured slowly into an NaOH/ice solution. The pH was adjusted to 10.
6. An extraction was made with petroleum ether. Ethanol was added to assist in phase separation and defoaming.
- 20 7. Product was dried by rotary evaporation followed by lyophilization.

EXAMPLE II

- 25 This Example illustrates the use of a chemically-modifiable thioether detergent in the assay for Neisseria gonorrhoeae, and compares the performance of the modifiable detergent to that of sodium dodecyl sulfate (SDS), using nitrocellulose for non-specific capture of
- 30 antigen and rabbit polyclonal antibodies for antigen detection.

35

Protocol

1. Antigen (Ag) Solubilization - N. gonorrhoeae (GC)
was solubilized (5×10^6 cells/ml) in
solubilization buffer consisting of:

Solubilization Buffer A = 0.5% Detergent I
($\text{NaO}_3\text{SO}(\text{CH}_2)_5\text{S}(\text{CH}_2)_5\text{CH}_3$) in 0.1 M Acetate
(pH 4.6) containing 0.1 M NaCl.

Solubilization Buffer B = 0.5% SDS
($\text{NaO}_3\text{SO}(\text{CH}_2)_{11}(\text{CH}_3)$) in 0.1 M Acetate
(pH 4.6) containing 0.1 M NaCl.

The solutions were incubated at room temperature for 15 minutes to promote solubilization of gonococcal cell surface components (Ag) and then further diluted 100 fold with the same buffers or with a detergent free buffer consisting of 0.1M acetate and 0.1M NaCl, pH 4.6. Controls consisted of the buffers alone without cells added.

2. Oxidation - To 0.5 ml of each of the solutions prepared from detergent containing buffer was added sufficient H_2O_2 (30%) to yield a final concentration of 1% after which the solution was incubated at room temperature for 15 minutes.

3. Assay Procedure

A. Aliquots (0.1 ml) of each solution were applied in duplicate to a nitrocellulose filter (0.8 μ pore size) mounted on an absorbent paper pad.

- 5 B. 0.1 ml Standard Diluent (80% fetal bovine serum, 15% glycerol, 4.9% 0.2M Tris (pH 7.5), and 0.1% Tween 20) was applied to the nitrocellulose filter to reduce non-specific binding of the remaining reagents.
- 10 C. 0.05 ml 1% whole Rabbit Anti-GC antiserum (in standard diluent) was then applied and allowed to incubate with the filter at room temperature for 5 minutes.
- 15 D. Washing was carried out by applying 0.1 ml of Standard Diluent to the filter.
- 20 E. 0.05 ml 1 µg/ml Horseradish peroxidase (HRP)-labeled goat anti-rabbit IgG (Heavy plus light chains) in Standard Diluent was applied and allowed to incubate with the filter at room temperature for 5 minutes.
- 25 F. Washing was carried out by applying 0.1 ml of Standard Diluent followed by 0.1 ml of 0.1 M citrate, pH 5.5.
- 30 G. 0.05 ml of a buffered solution comprising dicarboxidine and H_2O_2 , pH 5.0, was applied and incubation was carried out at room temperature for 8 minutes.
- 35 H. Reactions were stopped by addition of 0.1 mL of 0.1 M citrate, pH 5.5.
- I. Color intensity of the filter was recorded.

35 Results

The results are summarized in Table I.

TABLE I
Color Intensity
(Average of Two Results)

5	Detergent in dilution buffer	H ₂ O ₂	Without Ag	With Ag
	SDS	-	36	88
	SDS	+	33	104
	Detergent I	-	25	199
10	Detergent I	+	20	648
	None*	-	23	497
	None**	-	30	461

15 * prepared from GC solubilized with SDS

** prepared from GC solubilized with Detergent I

20 Conclusions

25 The assay response with GC diluted into 0.5% SDS is substantially less than when GC is diluted into detergent-free buffer and is not enhanced significantly by H₂O₂. Although the assay response with GC diluted into 0.5% Detergent I is greater than with SDS, it is still substantially less than when GC is diluted into detergent free buffer. After oxidation of detergent with H₂O₂ the assay response observed with 0.5% Detergent I is enhanced significantly and exceeds the response with 30 detergent-free buffer. It is concluded that either detergent interferes with binding of the antigen to the filter and that only in the absence of strong detergents can binding occur. Hydrogen peroxide converts Detergent I to a weak detergent and permits binding and therefore a 35 strong assay response.

EXAMPLE III

This example illustrates the use of a chemically-modifiable thioether detergent in an assay for Neisseria gonorrhoeae (GC), and compares the effects of the detergent in its modified and unmodified forms on assay sensitivity. Solid phase-immobilized rabbit anti-GC polyclonal antibodies were used for specific capture of antigen and mouse anti-GC monoclonal antibodies for antigen detection.

Protocol

1. Sample Pretreatment

A. N. gonorrhoeae was solubilized (1×10^6 cells/ml) in Dubecco's phosphate buffered saline (9mM phosphate, pH 7.2, 150mM NaCl) containing 1% Detergent I ($\text{CH}_3(\text{CH}_2)_5\text{S}(\text{CH}_2)_5\text{OSO}_3^-\text{Na}^+$), 0.1% Tween 20, and 2 mg/ml bovine serum albumin. The mixture was incubated for 15 minutes at room temperature.

B. H_2O_2 (30%) was added to 0.5 ml of the solubilized cells in a quantity sufficient to yield a final concentration of 1% and the mixture was incubated at room temperature for 15 minutes.

2. Assay Procedure

A. Aliquots (0.1 ml) of the solubilized cells before and after treatment with H_2O_2 were added to wells in a Suter-Butler coated microtiter plate (polystyrene coated in

- 5 succession with biotinylated-KLH,
streptavidin, and lastly with biotinylated
rabbit anti-GC) and allowed to stand for 1
hour at 37°C to promote the specific capture
of solubilized antigen on the solid
phase-immobilized rabbit anti-GC antibodies.
- 10 B. The samples were removed from the wells and
the wells were subsequently washed four times
with Dubecco's PBS containing 0.1% Tween-20.
The wells were filled to capacity during each
wash cycle.
- 15 C. The wells were next incubated for 1 hour at
37°C with 0.10 ml mouse anti-GC monoclonal
antibody (MAb 4G5) at 2 µg/ml in standard
diluent (50% fetal calf serum, 0.1% Tween 20,
50% Dubellco's PBS).
- 20 D. The solutions were removed from the wells and
the wells were subsequently washed four times
as indicated above in step B.
- 25 E. The wells were next incubated for 1 hour at
37°C with 0.1 ml HRP-labeled goat anti-mouse
IgG (0.12 µg/ml) in standard diluent.
- 30 F. The solutions were removed from the wells and
the wells were subsequently washed four times
as indicated above in step B.
- 35 G. The wells were then incubated for 30 minutes
at room temperature with 0.10 ml of a solution
of 0.83 mM 3,3',5,5'-tetramethyl
benzidine-dihydrochloride, 1 mM EDTA and 4 mM
urea peroxide in 50 mM citrate, pH 3.8.

H. Reactions were stopped by the addition of 0.1 ml in H_2SO_4 to each well.

5 I. Color intensity in each well was determined by measuring absorbance at 450 nm.

Results

	<u>Solubilization Treatment</u>	<u>Absorbance at 450 nm*</u>
10	H_2O_2 (1%)	1.81
	none	0.18

15 *Average of values obtained in duplicate assays. No Ag controls were run; absorbance values observed for such assays are generally in the range of 0.1 to 0.2.

Conclusion

20 The above data demonstrate that the assay interference associated with the presence of Detergent I in its native form is reduced substantially by treatment with H_2O_2 .

EXAMPLE IV

25 This example illustrates the use of a chemically-modifiable thioether detergent in the assay for Chlamydia trachomatis (CT), and compares the effects of the detergent in its modified and unmodified forms on
30 assay sensitivity using glass fiber for non-specific capture of antigen and rabbit polyclonal antibodies for antigen detection.

35

Protocol

1. Sample Pretreatment

- 5 A. Several Chlamydia-negative clinical swab
 samples (specimen collection site:human female
 urogenital tract) were solubilized with 1.0 ml
 0.1M acetate (pH 5.5) containing 1% Detergent
 I and incubating the mixtures at room
10 temperature for 15 minutes.
- B. The swabs were removed and the solutions were
 combined to yield a clinical sample pool.
- 15 C. Two aliquots (vol.=0.40 ml) of the clinical
 sample pool were combined with Chlamydia
 Elementary Bodies (EBs) that had been
 solubilized in 0.1 M acetate, 0.1 M NaCl, pH
 4.6 containing 0.5% Detergent I as described
20 for GC in Example II. Sufficient solubilized
 antigen was added to yield a final
 concentration equivalent to 1×10^5 EBs/ml.
 Controls consisted of the clinical sample pool
 alone without EBs added.
- 25 D. One aliquot was combined with sufficient
 H₂O₂ (30%) to yield a final concentration
 of 1%, and then incubated at room temperature
 for 15 minutes.

30 2. Assay Procedure

- A. A portion of each aliquot (0.05 ml) was
 applied to separate glass fiber filters (1.0 μ
35 pore size) mounted on absorbent paper pads.

- 5 B. 0.1 ml standard diluent (50% fetal bovine serum, 15% glycerol, 34.9% 0.01M Tris, pH 7.5, and 0.1% Tween 20 was added to each glass fiber filter.
- C. 0.05 ml 1% whole Rabbit Anti-CT antiserum (in standard diluent) was applied to each filter which was then allowed to stand at room temperature for 5 minutes.
- 10 D. 0.1 ml of the Standard Diluent was added to each filter.
- E. 0.05 ml 1 µg/ml Horseradish peroxidase (HRP)-labeled goat anti-rabbit IgG (Heavy plus light chains) in Standard Diluent was applied to each filter which was then allowed to stand at room temperature for 5 minutes.
- 15 F. Washing was then carried out by applying 0.1 ml Standard Diluent followed by 0.1 ml of citrate pH 5.5 to each filter.
- 20 H. 0.05 ml of a buffered solution of dicarboxidine and H_2O_2 , pH 5.5, was applied and incubation was carried out at room temperature for 8 minutes.
- 25 I. Reactions were stopped by addition of 0.1 M citrate, pH 5.5 (0.1 ml).
- 30 J. Color intensity was recorded at the reaction site.
- 35

Results

	<u>Solubilization Treatment</u>	<u>Color Intensity*</u>	
		+ EBs	- EBs
5	H ₂ O ₂ (1%)	993	1
	none	190	1

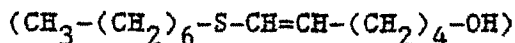
*arbitrary units

10 Conclusion

The above data demonstrate that the assay interference associated with the presence of Detergent I in its native form is reduced substantially by treatment with H₂O₂.

EXAMPLE V

Preparation of: 7-Thia-tetradecan-5-ene-1-ol



20 To 1.332 gr (10 mmol) of n-heptyl mercaptan and 981 mg (10 mmol) of 5-hexyn-1-ol dissolved in 25 ml of heptane under an argon atmosphere was added a few crystals of dibenzoyl peroxide. The reaction was stirred magnetically and heated to reflux for 18 hours. It was
25 then cooled and poured into 25 ml of 10% sodium bicarbonate and extracted with 3X25 ml of ethyl acetate. The organic phases were combined and dried over sodium sulfate, filtered, and evaporated to yield a yellow oil. The oil was chromatographed on eight silica gel type GF
30 preparative plates, using hexane-ethyl acetate (4:1) as developing solvent. Following removal of the band corresponding to product from the plates, the product was extracted from the silica with methanol, 684 mg (30%
35 yield) of pure 7-thia-tetradecan-5-ene-1-ol was obtained as a pale yellow oil.

^1H NMR (ppm, CDCl_3): 0.82(t, 3H, CH_3),
1.1-1.8(m, 14H, $-\text{CH}_2-$), 2.1(m, 2H, $-\text{CH}_2-\text{C}=\text{C}-$),
2.6(t, 2H, $-\text{CH}_2\text{S}-$), 3.62(t, 2H, $-\text{CH}_2\text{OH}$),
5.38-6.0(m, 2H, $-\text{CH}=\text{CH}-$).

5

EXAMPLE VI

Preparation of: Sodium 7-thiatetradecan-5-ene-1-ol
sulfate $(\text{CH}_3(\text{CH}_2)_6\text{S}-\text{CH}=\text{CH}-(\text{CH}_2)_4\text{OSO}_3\text{Na})$

10

To a stirred solution of 1.40 gr (10.4 mmol) of 7-thia-tetradecan-5-ene-1-ol from the previous reaction, in 10 ml of dry pyridine was added under an argon atmosphere 1.39 gr (10.4 mmol) of sulfur trioxide-trimethylamine complex. The reaction was heated to 15 50-60° for 15 minutes, then stirred overnight at room temperature. The reaction was monitored by thin layer chromatography (50% hexane-ethyl acetate: silica gel GF plates: iodine visualization) to confirm formation of the new, polar product. All pyridine was evaporated from the reaction, the product was dissolved in 10 ml of water, and 10 ml of a freshly prepared solution of 1 N sodium hydroxide was added dropwise with cooling. The colorless solution was frozen and lyophilized overnight; 1.66 gr 20 (77% yield) of sodium 7-thia-tetradecan-5-ene-1-ol sulfate was obtained as a white powder.

25

^1H NMR(ppm, D_2O): 0.70(t, 3H, CH_3),
0.9-1.8(br m, 14H, $-\text{CH}_2-$), 1.98(m, 2H, $-\text{CH}_2-\text{C}=\text{C}-$),
2.50(t, 2H, $-\text{CH}_2\text{S}-$), 3.91(t, 2H, $-\text{CH}_2\text{OSO}_3\text{Na}$),
30 5.30-5.95(m, 2H, $-\text{CH}=\text{CH}-$).

30

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity and understanding, it will be obvious 35 that certain changes and modifications may be practiced within the scope of the appended claims.

CLAIMS

1. A method for temporarily increasing the hydrophilic properties of a material, which method
5 comprises:

(a) contacting said material in an aqueous medium with a compound having detergent properties, said compound having the structural formula L-J wherein L is a lipophilic portion having a modifiable group, and J is a
10 hydrophilic portion, and wherein:

L is Y-A-Z:

wherein Y is alkyl, alkenyl, or alkynyl, having from 2 to 12 carbon atoms; or aralkyl, aralkenyl or aralkynyl, having
15 from 7 to 12 carbon atoms; A is -S-; -Se-; -CHX-, -CH(X)CH=CH- wherein X is Cl, Br, or I; -O-CH=CH-; -S-CH=CH- or -S-S-; and Z is saturated or unsaturated alkylene having from 1 to 10 carbon
20 atoms or saturated or unsaturated aralkylene having from 7 to 10 carbon atoms whereupon the hydrophilic properties of said material are increased; and

25 (b) modifying said modifiable group with a modifying reagent, whereupon said detergent properties are modified, and the hydrophilic properties of said material reduced.

30 2. The method of Claim 1 wherein said hydrophilic moiety J is an acidic group or a salt thereof, preferably wherein J is OSO_3H , SO_3H , COOH , PO(OR)OH , or OP(OR)OH , wherein R is H or lower alkyl.

35

3. The method of Claim 1 wherein the modifying step (b) includes the step of cleaving said compound having detergent properties with the modifying reagent, preferably wherein said cleaving is accomplished by using a nucleophile as the modifying reagent for step (b); most preferably wherein said nucleophile is a thiosulfate, a mercaptoacid, a thiourea, a hydroxylamine, a hydrazine, a hydrazide, or an ammonia.
4. The method of Claim 1 wherein the modifying step (b) includes the step of oxidizing said compound having detergent properties with the modifying reagent, preferably wherein said oxidizing is accomplished by using a peroxide, a peracid, a hypochlorite or hypobromite as the modifying agent for step (b); most preferably, the peroxide or peracid is a lower alkyl or acyl hydroperoxide, H_2O_2 , perborate, or persulfate.
5. The method of Claim 1 wherein the modifying step (b) includes the step of reducing said compound having detergent properties with the modifying reagent, preferably wherein said reducing is accomplished by using a sulfite, a hyposulfite salt, or a mercaptan as the modifying reagent for step (b).
6. The method of Claim 1 wherein said compound having detergent properties is a compound having the formula $CH_3(CH_2)_n-S-(CH_2)_mOSO_3H$, or a salt thereof, wherein n is an integer of 1 to 6, and m is an integer of 4 to 11, preferably wherein said compound has the formula $CH_3(CH_2)_2-S-(CH_2)_{11}OSO_3H$ or $CH_3(CH_2)_4-S-(CH_2)_6OSO_3H$.

7. A method of preparing an analyte for an assay, comprising the steps of:

(a) solubilizing the analyte with a detergent containing a modifiable group, said detergent having the structural formula L-J, wherein L is a lipophilic moiety, and J is a hydrophilic moiety; and

(b) modifying the properties of said detergent by the addition of a modifying reagent capable of modifying said modifiable group.

8. The method of Claim 7 using, as the detergent having the structure L-J for step (a), a compound wherein:

L is Y-A-Z:

wherein Y is alkyl, alkenyl, or alkynyl, having from 2 to 12 carbon atoms; or aralkyl, aralkenyl or aralkynyl, having from 7 to 12 carbon atoms;

A is -S-; -Se-; -CHX- or -CH(X)CH=CH-, wherein X is Cl, Br, or I; -O-CH=CH-; -S-CH=CH-; -S-S-;

-SO₂CH₂CH₂-O-;

a ketal group; or -Si(R)₂O- or -OSi(R)₂O- wherein R is independently H or lower alkyl; and

Z is saturated or unsaturated alkylene having from 1 to 10 carbon atoms or saturated or unsaturated aralkylene having from 7 to 10 carbon atoms, and

J is an acidic group or a salt thereof, preferably wherein J is OSO₃H, SO₃H, COOH, PO(OR)OH, or OPO(OR)OH, wherein R is H or lower alkyl.

9. A kit for conducting an assay for determining an analyte in a sample suspected of containing said analyte, wherein said analyte may be a member of a specific binding pair consisting of a ligand and its complementary receptor, said kit comprising in packaged form:

- (a) a compound having detergent properties and having the structural formula Y-A-Z-J, wherein: wherein Y is alkyl, alkenyl, or alkynyl, having from 2 to 12 carbon atoms; or aralkyl, aralkenyl or aralkynyl, having from 7 to 12 carbon atoms; A is -S-; -Se-; -CHX- or -CH(X)CH=CH-, wherein X is Cl, Br, or I; -O-CH=CH-; -S-CH=CH-; -S-S-; -SO₂CH₂CH₂-O-; a ketal group or -Si(R)₂O-; or -OSi(R)₂O- wherein R is independently H or lower alkyl; Z is saturated or unsaturated alkylene having from 1 to 10 carbon atoms or saturated or unsaturated aralkylene having from 7 to 10 carbon atoms;
- (b) J is a hydrophilic moiety; and
- (c) either one or both of: a modifying reagent capable of modifying said detergent properties of said compound Y-A-Z-J, or a member of a specific binding pair complementary to said analyte.

10. A composition comprising an aqueous medium containing a microbial antigen and/or its complementary antibody and a compound of the formula Y-A-Z-J, wherein: Y is alkyl, alkenyl, or alkynyl, having from 2 to 12 carbon atoms; or aralkyl, aralkenyl or aralkynyl, having from 7 to 12 carbon atoms;

A is -S-; -Se-; -CHX- or -CH(X)CH=CH-, wherein X is Cl, Br, or I; -O-CH=CH-; -S-CH=CH-; -S-S-;

5 -SO₂CH₂CH₂-O-; a ketal group; or -Si(R)₂O- or -OSi(R)₂O-; wherein R is independently H or lower alkyl;

 Z is saturated or unsaturated alkylene having from 1 to 10 carbon atoms or saturated or unsaturated aralkylene having from 7 to 10 carbon atoms; and

10 J is a hydrophilic moiety, preferably OSO₃H, SO₃H, COOH, PO(OR)OH, or OPO(OR)OH, wherein R is H or lower alkyl.

11. A compound having the formula
15 CH₃(CH₂)_n-A-(CH₂)_mOW, or a salt thereof, wherein A is -S- or -S-CH=CH-; W is H or SO₃H; and n is an integer of 2 to 4, and m is an integer of 6 to 11 when A is -S-, or n and m are independently 1 to 10 when A is -S-CH=CH-.

20

12. A method for temporarily increasing the hydrophilic properties of a material, substantially as described herein.

25

13. A method of preparing an analyte for an assay, substantially as described herein.

14. A kit for conducting an assay for determining an analyte in a sample suspected of containing said
30 analyte, substantially as described herein.

15. A composition, substantially as described herein.

35

16. A compound, substantially as described herein.